

anchoring protein anchoring domain interactions) (Goldenberg et al., *Journal of Nuclear Medicine*, 49(1):158-163 (2008)).

[0007] However, there remains a need for multimerized proteins, such as multimerized whole antibodies and antibody fragments (e.g., Fabs), which demonstrate enhanced biological and therapeutic properties. For example, there remains a need for multimerized proteins which have increased antibody avidity and enhanced antigen clustering compared to their non-multimerized counterparts.

SUMMARY

[0008] In one aspect, the present disclosure refers to a homodimer fusion protein comprising two antigen binding proteins, wherein said antigen binding proteins are linked to a transthyretin (TTR) protein complex, and wherein said antigen binding proteins are antibodies. In one example, the present disclosure relates to a homodimer fusion protein comprising two antigen binding proteins, wherein the antigen binding proteins are linked to a protein complex. In a particular example, the protein complex is a TTR protein complex. In a particular example, the antigen binding protein is an antibody. In another particular example, the antigen binding proteins are directly fused without a linker to the protein complex. In another particular example, the C-terminus of the antigen binding protein is directly fused to an N-terminus present in the TTR protein complex. In some examples, the antigen binding proteins are fused to the protein complex via a linker. In other examples, the C-terminus of the antigen binding protein is linked to an N-terminus present in the TTR protein complex. The linker may be an amino acid linker, such as an amino acid linker that is 1-20 amino acids in length. In particular examples, the amino acid linker is GGGGS, (GGGGS)₂, (GGGGS)₃, (GGGGS)₄, (GGGGS)₅, or (GGGGS)₆.

[0009] In one aspect, the present disclosure refers to a homotetramer fusion protein comprising four antigen binding proteins, wherein said antigen binding proteins are linked to a transthyretin (TTR) protein complex. In another example, the present disclosure relates to a homotetramer fusion protein comprising four antigen binding proteins, wherein the antigen binding proteins are linked to a protein complex. In one example, the protein complex is a TTR protein complex. In another example, the antigen binding protein is an antibody. The antigen binding protein may be a Fab. In some examples, the antigen binding proteins are directly fused without a linker to said protein complex. In particular examples, the C-terminus of the antigen binding protein is directly fused to an N-terminus present in the TTR protein complex. In other examples, the antigen binding proteins are fused to said protein complex via a linker. The C-terminus of the antigen binding protein may be linked to an N-terminus present in the TTR protein complex. In some examples, the linker is an amino acid linker, such as an amino acid linker that is 1-20 amino acids in length. In particular examples, the amino acid linker is GGGGS, (GGGGS)₂, (GGGGS)₃, (GGGGS)₄, (GGGGS)₅, or (GGGGS)₆.

[0010] In another aspect, the present disclosure also relates to a pharmaceutical composition comprising any of the homodimer or homotetramer fusion proteins discussed above.

[0011] In another aspect, the present disclosure relates to a use of the homodimer fusion protein as disclosed herein in the manufacture of a medicament for treating cancer, wherein said homodimer fusion protein comprises antigen binding proteins which bind to antigens on tumor cells. In yet another aspect, the present disclosure refers to a use of the homotetramer fusion protein as disclosed herein in the manufacture of a medicament for treating cancer, wherein said homodimer fusion protein comprises antigen binding proteins which bind to antigens on tumor cells. In addition, the present disclosure relates to a method of treating cancer using any of the homodimer or homotetramer fusion proteins discussed above. Moreover, the present disclosure relates to uses of any of the homodimer or homotetramer fusion proteins discussed above in the treatment of cancer. In another example, the present disclosure relates any of the homodimer or homotetramer fusion proteins discussed above for use in the treatment of cancer.

[0012] In another aspect, the present disclosure refers to a one or more isolated nucleic acid(s) encoding [a] a homodimer fusion protein as disclosed herein; or [b] a homotetramer fusion protein as disclosed herein. In another aspect, the present disclosure refers to an expression vector comprising a nucleic acid as disclosed herein. In another aspect, the present disclosure refers to a recombinant host cell comprising a nucleic acid as disclosed herein or the vector as disclosed herein. In some examples, the present disclosure relates to one or more isolated nucleic acid(s) encoding any of the homodimer or homotetramer fusion proteins discussed above. Expression vectors comprising such nucleic acids are also contemplated, as are recombinant host cells comprising the nucleic acids and/or vectors discussed herein. In particular examples, the recombinant host cell is a Chinese hamster ovary (CHO) cell, E5 cell, baby hamster kidney (BHK) cell, monkey kidney (COS) cell, human hepatocellular carcinoma cell, or human embryonic kidney 293 (HEK 293) cell.

[0013] In another aspect, the present disclosure refers to a method of making a homodimer fusion protein as disclosed herein or a homotetramer fusion protein as disclosed herein, said method comprising: a) culturing the recombinant host cell as disclosed herein; and b) isolating the homodimer or homotetramer fusion protein from said culture. Methods of making any of the homodimer or homotetramer fusion proteins discussed above are also part of the present disclosure. Such methods may comprise a) culturing the recombinant host cell of claim 32 or 33; and b) isolating the homodimer or homotetramer fusion protein from said culture.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIG. 1. Figure 1 is a schematic representation of the homomultimer constructs of the present invention. Figure 1a is an exemplary TTR antibody homodimer fusion protein, where the C-terminus of both antibody heavy chains is linked to the N-terminus of each TTR subunit. Figure 1b is an exemplary TTR antibody homotetramer fusion protein, where one of the two heavy chains of each antibody C-terminus is linked to the N-terminus of each TTR subunit. The “+” and “-” signs indicate Fc charge pairs which allow for consistent attachment of one TTR subunit per whole antibody. Figure 1c is an exemplary TTR Fab homotetramer fusion protein, where the C-terminus of each Fab fragment is linked to the N-terminus of each TTR subunit. Each of Figures 1a-1c shows the optional linker between the heavy chain and TTR.

[0015] FIG. 2. Figure 2 is a series of SDS-PAGE gels that demonstrate that the anti-CB1 TTR antibody homodimer with no linker and varying linker lengths (Figure 2a), anti-CB1 TTR antibody homotetramer with no linker (Figure 2b), and anti-CB1 TTR Fab homotetramer proteins with no linker (Figure 2c), respectively, are robustly expressed in HEK 293 cells. Figure 2 is further discussed in Example 2.

[0016] FIG. 3. Figure 3 is a series of HPLC size exclusion chromatography (SEC) analyses of the anti-CB1 TTR antibody homodimer fusion protein with no linker, a (G₄S) linker, a (G₄S)₂ linker, a (G₄S)₃ linker, or a (G₄S)₄ linker. Figure 3 is further discussed in Example 2.

[0017] FIG. 4. Figure 4 shows that the TTR anti-CB1 antibody homotetramer and TTR anti-CB1 Fab homotetramer fusion proteins improve EC₅₀ compared to parental CB1 Ab. Figure 4 is further discussed in Example 3.

[0001] FIG. 5. Figure 5 is an SDS-PAGE gel that demonstrates that the anti-GITR TTR antibody homodimer (Lane 1), anti-GITR TTR antibody homotetramer (Lane 2), and anti-GITR TTR Fab homotetramer proteins (Lane 3), respectively, are expressed in HEK 293 cells. Lanes 4-7 are anti-dinitrophenyl (anti-DNP) antibody. Figure 5 is further discussed in Example 4.

[0042] The term “antibody” refers to an immunoglobulin of any isotype. An “antibody” is a type of antigen binding protein. The term “antibody” includes, but is not limited to, monoclonal antibodies, human antibodies, humanized antibodies, chimeric antibodies, and anti-idiotypic (anti-Id) antibodies. Antibodies can be of any isotype/class (e.g., IgG, IgE, IgM, IgD, IgA and IgY), or subclass (e.g., IgG1, IgG2, IgG3, and IgG4). In some embodiments, an antibody comprises at least two full-length heavy chains and two full-length light chains. In other embodiments, an antibody includes fewer chains such as antibodies naturally occurring in camelids which may comprise only heavy chains. Antibodies may be derived solely from a single source, or may be “chimeric” wherein different portions of the antibody are derived from two different antibodies as described further below. The antigen binding proteins, antibodies, or binding fragments may be produced, for example, in hybridomas, by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact antibodies.

[0043] The term “antigen” refers to a molecule or a portion of a molecule capable of being bound by a binding agent, such as an antigen binding protein (including, e.g., an antibody), and additionally capable of being used in an animal to produce antibodies capable of binding to that antigen. An antigen may possess one or more epitopes that are capable of interacting with different antigen binding proteins, e.g., antibodies.

[0044] An “antigen binding protein” as used herein means any protein that specifically binds a specified target antigen. The term includes polypeptides that include at least one antigen binding region. The term also encompasses antibodies that comprise at least two full-length heavy chains and two full-length light chains, as well as derivatives, variants, fragments, and mutations thereof. An antigen binding protein also includes Fab, Fab’, F(ab’)₂, Fv fragments, domain antibodies such as Nanobodies® and single-chain antibodies, as described in more detail below.

[0045] An “antigen binding region” or “antigen binding domain” means the portion of a protein, such as an antibody or a fragment, derivative, or variant thereof, that specifically binds to, interacts with, or recognizes a given epitope or site on a molecule (e.g., an antigen). For example, the portion of an antigen binding protein that contains the amino acid residues that interact with an antigen and confer on the antigen binding protein its specificity and affinity for the antigen is referred to as “antigen binding region.” An antigen binding region can include one or more “complementarity determining regions” (“CDRs”). Certain antigen binding regions also include one or more “framework” regions. “Framework” regions can contribute directly to the specific binding of the antigen binding protein, but typically aid in maintaining the proper conformation of the CDRs to promote binding between the antigen binding region and an antigen.

[0046] The terms “cancer”, “tumor”, “cancerous”, and “malignant” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma including adenocarcinoma, lymphoma, blastoma, melanoma, sarcoma, and leukemia. More particular examples of such cancers include

What is claimed:

1. A homodimer fusion protein comprising two antigen binding proteins, wherein said antigen binding proteins are linked to a transthyretin (TTR) protein complex, and wherein said antigen binding proteins are antibodies.
2. The homodimer fusion protein of claim 1, wherein said antigen binding proteins are directly fused without a linker to said TTR protein complex.
3. The homodimer fusion protein of claim 2, wherein said C-terminus of the antigen binding proteins is directly fused to an N-terminus present in the TTR protein complex.
4. The homodimer fusion protein of claim 1, wherein said antigen binding proteins are fused to said TTR protein complex via a linker.
5. The homodimer fusion protein of claim 4, wherein said C-terminus of the antigen binding proteins is linked to an N-terminus present in the TTR protein complex.
6. The homodimer fusion protein of claim 5, wherein said linker is an amino acid linker.
7. The homodimer fusion protein of claim 6, wherein said amino acid linker is 1-40 amino acids in length.
8. The homodimer fusion protein of claim 7, wherein said amino acid linker is GGGGS, (GGGGS)₂, (GGGGS)₃, (GGGGS)₄, (GGGGS)₅, or (GGGGS)₆.
9. A homotetramer fusion protein comprising four antigen binding proteins, wherein said antigen binding proteins are linked to a transthyretin (TTR) protein complex.
10. The homotetramer fusion protein of claim 9, wherein the antigen binding protein is an antibody.
11. The homotetramer fusion protein of claim 9, wherein the antigen binding protein is a Fab.
12. The homotetramer fusion protein of any one of claims 9-11, wherein said antigen binding proteins are directly fused without a linker to said protein complex.

13. The homotetramer fusion protein of claim 12, wherein said C-terminus of the antigen binding proteins is directly fused to an N-terminus present in the TTR protein complex.
14. The homotetramer fusion protein of any one of claims 9-11, wherein said antigen binding proteins are fused to said protein complex via a linker.
15. The homotetramer fusion protein of claim 14, wherein said C-terminus of the antigen binding proteins is linked to an N-terminus present in the TTR protein complex.
16. The homotetramer fusion protein of claim 15, wherein said linker is an amino acid linker.
17. The homotetramer fusion protein of claim 16, wherein said amino acid linker is 1-40 amino acids in length.
18. The homotetramer fusion protein of claim 17, wherein said amino acid linker is GGGGS, (GGGGS)₂, (GGGGS)₃, (GGGGS)₄, (GGGGS)₅, or (GGGGS)₆.
19. A pharmaceutical composition comprising the homodimer fusion protein of any one of claims 1-8.
20. A pharmaceutical composition comprising the homotetramer fusion protein of any one of claims 9-18.
21. Use of the homodimer fusion protein of any one of claims 1-8 in the manufacture of a medicament for treating cancer, wherein said homodimer fusion protein comprises antigen binding proteins which bind to antigens on tumor cells.
22. Use of the homotetramer fusion protein of any one of claims 9-18 in the manufacture of a medicament for treating cancer, wherein said homodimer fusion protein comprises antigen binding proteins which bind to antigens on tumor cells.
23. One or more isolated nucleic acid(s) encoding [a] a homodimer fusion protein of any one of claims 1-8; or [b] a homotetramer fusion protein of any one of claims 9-18 .
24. An expression vector comprising a nucleic acid of claim 23.

25. A recombinant host cell comprising a nucleic acid of claim 23 or the vector of claim 24.

26. The recombinant host cell of claim 25, wherein said host cell is a Chinese hamster ovary (CHO) cell, E5 cell, baby hamster kidney (BHK) cell, monkey kidney (COS) cell, human hepatocellular carcinoma cell, or human embryonic kidney 293 (HEK 293) cell.

27. A method of making a homodimer fusion protein of any one of claims 1-8 or a homotetramer fusion protein of any one of claims 9-18, said method comprising:

- a) culturing the recombinant host cell of claim 25 or 26; and
- b) isolating the homodimer or homotetramer fusion protein from said culture.